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AN INVESTIGATION OF PUTATIVE *TRAGOPOGON MIRUS* (ASTERACEAE) POPULATIONS IN OREGON, USA

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Abstract

Tragopogon mirus is a recently formed allotetraploid species, with *Tragopogon dubius* and *Tragopogon porrifolius* as the parental species. A study was conducted on *Tragopogon* populations in Oregon, USA to determine if *T. dubius* and *T. porrifolius* hybridization and subsequent polyploid speciation had occurred. We examined ploidy levels as well as nucleotide data from the chloroplast *trnT-trnL* spacer and nuclear ribosomal ITS regions. While no hybridization or polyploid speciation was found, a seven base pair length mutation found in the chloroplast *trnT-trnL* spacer region should prove useful for determining parentage in populations of *T. mirus.*

Key Words: Oregon, cpDNA, ITS, molecular marker, polyploidy, *Tragopogon mirus.*

Tragopogon L. is an Eurasian genus of 20 species. Three species of this genus, *Tragopogon dubius* Scop., *Tragopogon porrifolius* L., *and Tragopogon pratensis* L. were introduced to North America near the beginning of the twentieth century (Ownbey 1950; Novak et al. 1991). In the late 1940s Marion Ownbey discovered several hybrid, allopolyploid populations of these species in the Palouse region of Washington and Idaho. Ownbey (1950) described and named the allotetraploid species *Tragopogon mirus,* whose parents are *T. dubius* and *T. porrifolius,* and *Tragopogon miscellus,* whose parents are *T. dubius* and *T. pratensis.* In subsequent years more populations of *T. mirus* have been found in the Palouse region (Soltis and Soltis 1991) and Arizona (Brown and Schaak 1972). *Tragopogon mirus* has also been reported in Oregon (Chambers and Sundberg 2000) based on one specimen at the Oregon State University Herbarium (*Peck 22380*). Studies conducted over the past fifty years suggest at least nine independent origins of *T. mirus* (Ownbey and McCollum 1953, 1954; Brehm and Ownbey 1965; Soltis and Soltis 1989; Novak et al. 1991; Soltis and Soltis 1991; Soltis et al. 1995; Cook et al. 1998).

Our study was motivated by a population of putative *Tragopogon* hybrids, located in the city of Corvallis in the Willamette Valley of western Oregon. The plants of the population, which number approximately 100, resemble *T. porrifolius* except in ligule color. While nearby *T. porrifolius* populations exhibit dark purple ligules, the Corvallis population has light purple ligules, appearing an intermediate color between *T. porrifolius* and yellow-liguled *T. dubius.* Furthermore, the Corvallis site is a disturbed site with sympatric *T. porrifolius* and *T. dubius* populations.

A study of several allopolyploid *Tragopogon* populations, in the Palouse region (Soltis and Soltis 1989), revealed that the maternal and paternal parent of *T. mirus* were always *T. porrifolius* and *T. dubius,* respectively. The authors of the study attribute this one sided parentage to the fact that, in the Palouse region, *T. dubius* is more common and widespread than *T. porrifolius.* They concluded that pollen load may be an important factor in determining parentage. In ligule color, plants in the Corvallis population differ from known *T. mirus* populations. *Tragopogon mirus* typically has bicolored ligules, with the center yellow and the outer ligules purple. Plants of the Corvallis population have solid, light purple ligules. We postulated that this differing morphology might be due to reversed parentage. In the Willamette Valley *T. porrifolius* is more common and widespread than *T. dubius* (Oregon Flora Project 2004).

In addition to the putative hybrids in Corvallis, we examined two herbarium specimens from northeastern Oregon: *Stevenson s.n.* (ORE) which matched plants of the Corvallis population in ligule color and the putative *T. mirus, Peck 22380* (WILLU), reported by Chambers and Sundberg (2000) (Table 1).

To evaluate hybridization and parentage we sequenced both the plastid *trnT-trnL* spacer and nuclear ribosomal ITS regions. Ploidy levels were measured using chromosome counts and flow cytometry.

Materials and Methods

DNA was obtained from fresh plant material, dried herbarium specimens and cotyledons germi-

Table 1. Collection Data and Plant Material Used for DNA Extractions and Subsequent Determinations of Putative Hybrid Specimens.

Sample	Collection data (all locations in Oregon)	DNA obtained from	Determination from DNA data
Known *T. dubius*			
D1	Benton Co., Corvallis, *Schenk 118-1* (OSC)	fresh leaf	
D2	Benton Co., Corvallis, *Schenk 118-2* (OSC)	fresh leaf	
Known *T. porrifolius*			
P1	Benton Co., Corvallis, *Meyers 112* (OSC)	fresh leaf	
P2	Benton Co., Corvallis, *Meyers 113* (OSC)	fresh leaf	
Putative hybrids			
M1	Benton Co., Corvallis, *Meyers 111* (OSC)	fresh leaf	*T. porrifolius*
MC	Benton Co., Corvallis, *Halse 5890* (OSC)	seed	*T. porrifolius*
ORE	Union Co., near La Grande, *Stevenson s.n.* (ORE)	dried leaf	*T. porrifolius*
WILLU	Union Co., near Elgin, *Peck 22380* (WILLU)	dried leaf	*T. dubius*

nated from seed (Table 1). Approximately 50 mg of plant material was used to extract DNA using a DNeasy Plant Mini kit (Qiagen, Valencia, CA). Polymerase chain reactions for plasmid DNA were performed in 20 µl reactions volumes which contained, 3.0 mM $MgCl_2$ (Promega, Madison, WI), 10X Promega Buffer A, 0.1 µM each dATP, dCTP, dGTP and dTTP (Epicentre, Madison, WI), 10 pmol of each primer (Taberlet et al. 1991), 1X BSA, 1 unit of *Taq* polymerase (Promega), and 2.0 µl DNA template. ITS reactions were conducted using the same amount and concentration of reactants with the following exceptions: 5% dimethysulfoxide (DMSO) and 10 pmol each primer (Liston et al. 1996). The reactions, overlaid with approximately 10 µl of mineral oil, were placed in an MJ research programmable thermal controller programmed as follows: 5 min at 95°C, 25 cycles of 1 min at 95°C, 4 min at 65°C, with a 10 min final extension at 65°C. Following PCR, products were purified using a QIAquick PCR purification kit (Quigen, Valencia, CA). As a result of low DNA yields the 50 µl elutions were concentrated to 5 µl using a LABCONCO centrivap-concentrator. DNA sequences were obtained using an ABI 373A DNA sequencer (Applied Biosystems, Foster City, CA). Sequences were aligned and analyzed using BioEdit for Windows 95/98/NT (Hall 1999).

Chromosome counts were performed on putative hybrids grown from seeds collected at the Corvallis site, using a modified procedure described by Riera-Lizarazu et al. (1996). Root tips were collected in the morning (10:30 a.m.) and placed in a flask with a pre-treatment solution of 0.5 g $liter^{-1}$ colchicine, 25 g $liter^{-1}$ 8-hydroxyquinoline, and 1.5% (v/v) DMSO for 3.5 h at room temperature in the dark. Root tips were transferred to a solution of 2% (w/v) aceto orcein solution and stored in a refrigerator for 48 h. Root tips were squashed in 45% acetic acid and the chromosomes counted.

Flow cytometry was performed on putative hybrid cotyledons grown from seeds collected at the Corvallis site using a Partec GmbH ploidy analyzer (Partec, Münster, Germany).

Results

The chromosome counts of putative hybrids, grown from seed collected at the Corvallis site, revealed a chromosome number of $2n=12$. To confirm the chromosome counts we compared the ploidy level of several other putative hybrids with the known ploidy level of *T. dubius* and *T. porrifolius* ($2n=12$) using flow cytometry. On the cytometer, the putative hybrids produced peaks in the same channel as *T. dubius* and *T. porrifolius*. From these results, we conclude the plants in the Corvallis population are diploid.

To explore the possibility of hybridization we examined sequence data of the chloroplast *trnT-trnL* spacer and nuclear ribosomal ITS regions. Upon alignment, *trnT-trnL* sequences of *T. porrifolius* and *T. dubius* populations revealed three base pair changes and a seven base pair length mutation (GenBank acc. nos. AY525374, AY525375). The ITS sequences revealed seven base pair changes (GenBank acc. nos. AY525376, AY525377). These distinct sequences were compared with those of the putative hybrids. The sequences of three putative hybrids, including the Corvallis population, were found to match the sequences of *T. porrifolius,* while sequences of the herbarium specimen from Elgin (*Peck 22380*) were found to match sequences of *T. dubius* (Table 1).

Discussion

The seven base pair length mutation in the plastid *trnT-trnL* spacer region clearly distinguishes the chloroplast genomes of *T. dubius* and *T. porrifolius* and can be easily scored on an agarose gel. The chloroplast genome is maternally inherited in most angiosperms, including members of the Asteraceae (Sears 1980; Whately 1982; Corriveau and Coleman 1988). Further, cytological and cpDNA studies suggest that the chloroplast genome is maternally

inherited in *Tragopogon* (Ownbey and McCollum 1953; Soltis and Soltis 1989). In ongoing and future studies, the seven base pair length mutation should provide an effective marker to determine parentage in populations of *T. mirus.*

Chromosome counts and flow cytometry confirm that plants at the Corvallis site are diploid, and not tetraploid, as in *T. mirus.* In addition, DNA sequences confirm that the Corvallis plants and the herbarium specimens analyzed are *T. porrifolius.* The result of this study is that no Oregon records of *T. mirus* were found, including the specimen cited by Chambers and Sundberg (2000). We conclude the light colored ligules of the plants and specimens we analyzed are not the result of a hybridization event between *T. dubius* and *T. porrifolius,* but rather a morphological variant of typical *T. porrifolius.*

Despite sympatric populations of *T. dubius* and *T. porrifolius,* hybridization and subsequent alloploid speciation of *T. mirus* has yet to be recorded in Oregon. One difference between the Palouse region of Washington and Idaho, where *T. mirus* is commonly found, and the Willamette Valley, is the asymmetric abundance of *T. dubius* and *T. porrifolius.* In the Palouse region, *T. dubius* is more common than *T. porrifolius,* while in the Willamette Valley the respective abundance is opposite. Given that *T. dubius* has always been found to be the paternal parent of *T. mirus* in the Palouse region (Soltis and Soltis 1989), the lesser abundance of *T. dubius* in the Willamette Valley, and resulting lower pollen contribution, may explain the absence of *T. mirus* in western Oregon. In northeastern Oregon, *T. dubius* is abundant while *T. porrifolius* is very rare and only known from the vicinity of La Grande (Oregon Flora Project 2004). Although the two examined herbarium specimens were determined to not represent *T. mirus,* field studies are needed to confirm the absence of this hybrid species in the region.

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Literature Cited

Brehm, B. G. and M. Ownbey. 1965. Variation in chromatographic patterns in the *Tragopogon dubius-pratensis-porrifolius* complex (Compositae). American Journal of Botany 52:811–818.

Brown, R. K. and C. G. Schaak. 1972. Two new species of *Tragopogon* for Arizona. Madroño 21:304.

Chambers, K. L. and S. Sundberg. 2000. Oregon vascular plant checklist: Asteraceae, 2nd ed. Oregon Flora Project, Oregon State University, Corvallis, OR.

Cook, L. M., P. S. Soltis, S. J. Brunsfeld, and D. E. Soltis. 1998. Multiple independent formations of *Tragopogon* tetraploids (Asteraceae): evidence from RAPD markers. Molecular Ecology 7:1293–1302.

Corriveau, J. L. and A. W. Coleman. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. American Journal of Botany 75: 1443–1458.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Liston, A., W. A. Robinson, J. M. Oliphant, and E. Alvarez-Buylla. 1996. Length variation in the nuclear ribosomal DNA internal transcribed spacers of nonflowering seed plants. Systematic Botany 21:1–12.

Novak, S. J., D. E. Soltis, and P. S. Soltis. 1991. Ownbey's Tragopogons: forty years later. American Journal of Botany 78:1586–1600.

Oregon Flora Project. 2004. Oregon plant atlas. Available at: http://www.oregonflora.org/oregonplantatlas.html. Oregon Flora Project, Oregon State University, Corvallis, OR.

Ownbey, M. 1950. Natural hybridization and amphiploidy in the genus *Tragopogon.* American Journal of Botany 37:487–499.

——— and G. McCollum. 1953. Cytoplasmic inheritance and reciprocal amphiploidy in *Tragopogon.* American Journal of Botany 40:788–796.

——— and ———. 1954. The chromosomes of *Tragopogon.* American Journal of Botany 56:7–21.

Riera-Lizarazu, O., H. W. Rines, and R. L. Phillips. 1996. Cytological and molecular characterization oat × maize partial hybrids. Theoretical and Applied Genetics 93:123–135.

Sears, B. B. 1980. Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. Plasmid 4:233–255.

Soltis, D. E. and P. S. Soltis. 1989. Allopolyploid speciation in *Tragopogon*: insights from chloroplast DNA. American Journal of Botany 76:119–124.

Soltis, P. S. and D. E. Soltis. 1991 Multiple origins of the allotetraploid *Tragopogon mirus* (Compositae): rDNA evidence. Systematic Botany 16:407–413.

———, G. M. Plunkett, S. J. Novak, and D. E. Soltis. 1995. Genetic variation in *Tragopogon* species: additional origins of the allotetraploids *T. mirus* and *T. miscellus* (Compositae). American Journal of Botany 82:1329–1341.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17:1105–1109.

Whatley, J. M. 1982. Ultrastructure of plastid inheritance: green plants to angiosperms. Biological Reviews 57:527–569.